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Mosaicism for del(17)(p11.2p11.2) Underlying the Smith-Magenis Syndrome

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Smith-Magenis syndrome (SMS) is a multiple congenital anomalies/mental retardation syndrome associated with deletion of band p11.2 of chromosome 17. The deletion is typically detected by high-resolution cytogenetic analysis of chromosomes from peripheral lymphocytes. Fluorescence in situ hybridization (FISH) has been previously used to rule out apparent mosaicism for del(17)(p11.2p11.2) indicated by routine cytogenetics. We now report mosaicism for del(17)(p11.2p11.2) in a child with SMS. The mosaicism had gone undetected during previous routine cytogenetic analysis. FISH analysis of peripheral lymphocytes as well as immortalized lymphoblasts using markers from 17p11.2 revealed that approximately 60% of cells carried the deletion. To our knowledge, this is the first case of SMS associated with mosaicism for del(17)(p11.2p11.2).

KEY WORDS: **mosaicism, chromosome 17p11.2, Smith-Magenis syndrome, FISH, del(17)(p11.2p11.2)**

INTRODUCTION

Smith-Magenis syndrome (SMS) is a multiple congenital anomalies/mental retardation syndrome characterized by an interstitial deletion on the short arm of chromosome 17 involving band p11.2 [Smith et al., 1982, 1986; Stratton et al., 1986; Greenberg et al., 1991]. Greenberg et al. [1991] have assessed 31 SMS patients and reported the most common physical and clinical findings. Physical findings include flat midface, brachycephaly, broad nasal bridge, and brachydactyly. Behavioral and functional anomalies include mental retardation, short stature, failure to thrive, hyperactivity, hoarse deep voice, self-destructive behavior, and sleep disturbances. Ocular pathologies of high myopia and retinal detachments [Finucane et al., 1993a] and a characteristic spasmodic upper body squeeze or "self-hugging behavior" have also been noted in SMS patients [Finucane et al., 1994]. Molecular studies on SMS patients have defined a common deletion region in most SMS patients [Greenberg et al., 1991; Juyal et al., 1996] and several candidate genes have been identified [Chevillard et al., 1993; Chen et al., 1995; Zhao et al., 1995; Elsea et al., 1995] but further investigation is required to define the genetic cause of SMS.

Mosaicism for del(17)(p11.2p12) has been reported and proven using molecular techniques in a single SMS case to date [Zori et al., 1993]. However, this individual was apparently unaffected and the mosaic condition uncovered due to a retrospective chromosome analysis conducted because she gave birth to a child with SMS. The mosaicism (55%) was apparent at low resolution (<500 bands) and was further demonstrated by construction of somatic cell hybrids retaining the individual chromosome 17 homologues and molecular analysis of these somatic cell hybrids. Two other cases, diagnosed as mosaic del(17)(p11.2p11.2) by routine cytogenetic analysis [Juyal et al., 1995a; Finucane et al., 1993b], were shown to carry a deletion in all cells by fluorescence in situ hybridization (FISH) analysis [Juyal et al., 1995a,b]. We now report a case diagnosed as del(17)(p11.2p11.2) in previous cytogenetic analysis who is mosaic for del(17)(p11.2p11.2) when examined by FISH analysis. This is the first report of a patient who is mosaic for del(17)(p11.2p11.2) and displays the characteristic SMS phenotype.

METHODS

The patient HOU121-492 was 1 of 62 SMS patients with a cytogenetic diagnosis of an interstitial deletion involving band p11.2 of chromosome 17 referred for molecular analysis of the deletion. The details of this analysis are presented elsewhere [Juyal et al., 1996]. Forty-five of these patients were evaluated by fluorescence in situ hybridization (FISH) as described previously by Juyal et al. [1995a] using the cosmid probes c142F7 [Juyal et al., 1995a] and c105G12 [Juyal et al.,

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1996] from 17p11.2 and the control probe c123F8 from the thymidine kinase locus on 17q [Juyal et al., 1995a]. Genotyping of microsatellite loci was conducted as described previously [Figuera et al., 1995].

RESULTS

The patient, HOU121-492, was described by Kondo et al. [1991] (patient 3 in that report) as having the physical attributes of SMS with delayed language development, attention problems, and hyperactivity. High resolution G-banding analysis was conducted on cultured peripheral blood lymphocytes and a partial deletion of band 17p11.2 was noted. We undertook FISH analysis of Epstein-Barr virus transformed lymphoblasts from HOU121-492 using various markers in 17p11.2 in conjunction with a control cosmid c123F8 representing the thymidine kinase locus on 17q to evaluate the extent of the deletion. Using the cosmid c142F7 representing the locus D17S258 which is deleted in all SMS patients examined to date [Juyal et al., 1996], a single hybridization signal was noted in 43/72 metaphases examined (60%) (Fig. 1b). Both chromosome 17 homo-

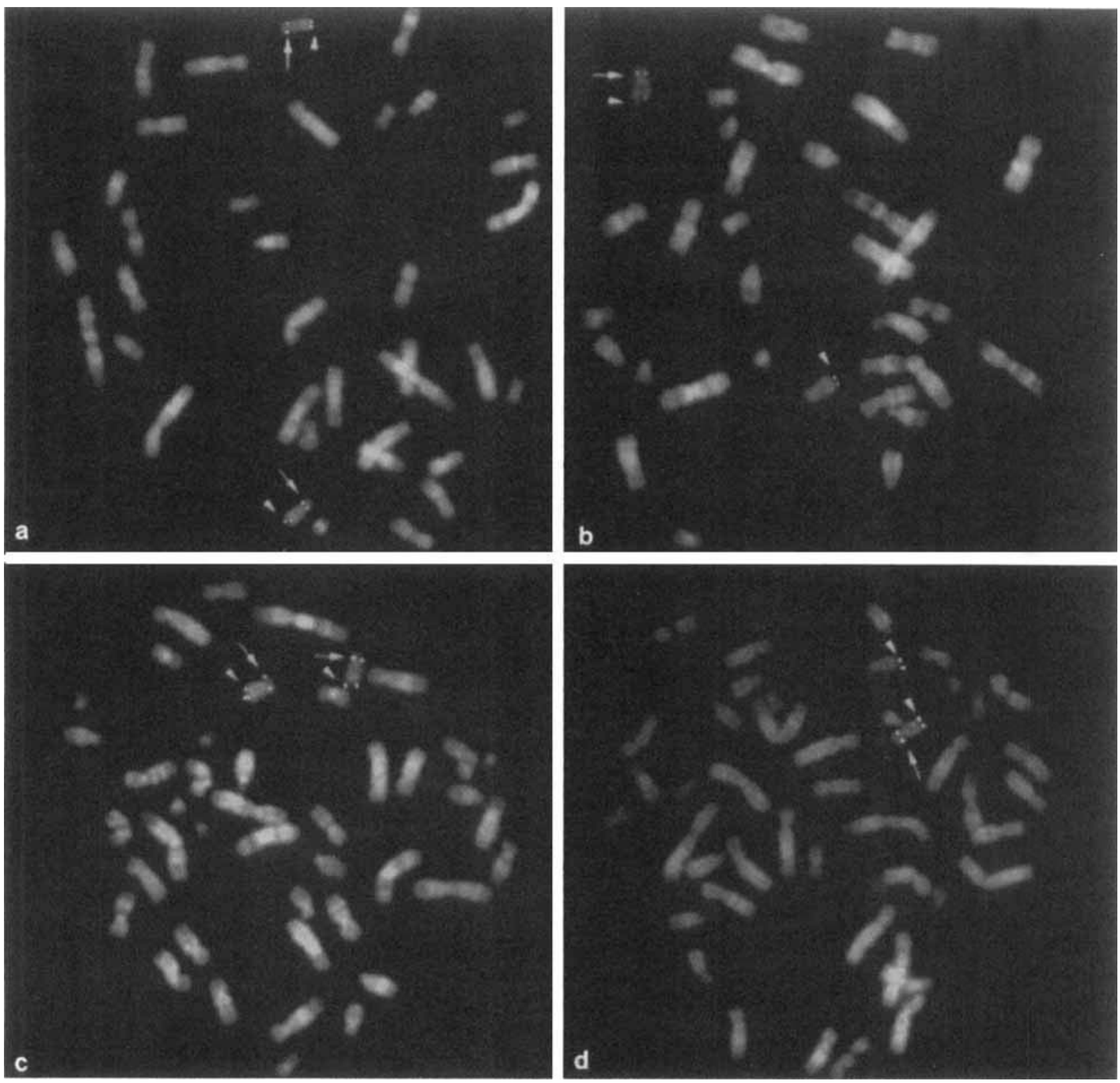

Fig. 1. FISH results on metaphase chromosomes from lymphoblasts (**a** and **b**) or lymphocytes (**c** and **d**) of patient HOU121-492 hybridized in situ with biotinylated test probe c142F7 along with a control probe c123F8. Representative data indicating mosaicism for del(17)(p11.2p11.2) are shown. In a and c, hybridization signals from cosmid c142F7 (arrow) are present on the short arm of both chromosome 17 homologues while in b and d, signal from cosmid c142F7 is present on the short arm of only one chromosome 17 homologue. Hybridization signals from the control cosmid c123F8 (arrowhead) are present on the long arm of both chromosome 17 homologues.

logues showed a signal in 29/72 metaphases examined (40%) (Fig. 1a). Similarly, using cosmid c105G12 representing the locus D17S446 which is deleted in the majority of SMS patients examined [Juyal et al., 1996], the deletion was seen in 58/92 metaphases (63%) examined. Thus, approximately 60% of the lymphoblastoid cells carried the deletion on one chromosome 17 homologue, while approximately 40% had no deletion, indicating mosaicism for del(17)(p11.2p11.2) in the patient.

To rule out contamination of the cell line with a normal cell line as a possible cause for the mosaicism, we conducted genotyping at four different highly polymorphic loci (heterozygozity >0.70) using DNA from the lymphoblastoid lines of the patient, HOU121-492, and her mother, HOU121-493. The results shown in Figure 2 indicate that the patient had only one or two alleles at each of the loci which would be unexpected if the cell line were contaminated with another cell line bearing two normal chromosome 17 homologues.

To further confirm the mosaicism, a fresh peripheral blood sample from the patient HOU121-492 was used to prepare chromosomes which were analyzed by FISH. The results obtained were similar to those obtained using lymphoblastoid cells; 31/49 metaphases examined with c142F7 (D17S258) demonstrated a deletion on one chromosome 17 homologue indicating that 63% of cells carried the deletion (Fig. 1d), while 37% did not (Fig. 1c).

DISCUSSION

This is the first report of mosaicism for del(17)(p11.2p11.2) leading to the SMS phenotype. A few cases of somatic mosaicism for other chromosomal deletions have been previously reported. Somatic mosaicism for 18q- in association with Rett syndrome [Gordon et al., 1993], for deletion of 13q in patients with retinoblastoma [Michalova et al., 1982; Dudin et al., 1984], for 5q- in cri du chat patients [Romano et al., 1991] and for del(15)(q11-13) in Prader-Willi syndrome patients [Cassidy et al., 1984] have been previously noted. In addition, FISH studies provided evidence for somatic mosaicism in mothers of boys with de novo dystrophin gene deletions [Bunyan et al., 1995; Voit et al., 1992]. Mosaicism could pose a significant problem in attempting to establish a phenotype-genotype correlation as it may be a tissue-specific phenomenon. Mosaicism observed in a particular tissue may not have any bearing on any phenotype, or mosaicism in a tissue manifesting the phenotype may escape detection. Since occurrence of somatic mosaicism is a purely post-zygotic phenomenon, severity of its effect depends upon the time of its onset during development, the degree of mosaicism and the tissues affected. This may explain the difference in phenotypes between the mosaic case reported previously [Zori et al., 1993] and the patient HOU121-492 in whom we identified evidence for mosaicism in this study.

The extent of the deletion in HOU121-492 was similar to that seen in most SMS patients examined to date [Juyal et al., 1996], with a proximal breakpoint between markers D17S58 and D17S446 and a distal breakpoint between markers cCI17-638 and cCI17-498 [Juyal et al., 1996]. This study illustrates that routine cytogenetic analysis can fail to detect mosaicism even when the deletion is of the "typical" size. In the previous studies where mosaicism was diagnosed cytogenetically but ruled out by FISH analysis [Juyal et al., 1995a; Finucane et al., 1993b; Juyal et al., 1995b], the deletions were smaller than the typical SMS deletion, thus, explaining why some metaphases may have been scored as "normal" in karyotype analysis. In contrast, in the case where mosaicism for del(17)(p11.2p12) was diagnosed cytogenetically and proven molecularly, both cytogenetic and molecular analyses indicated that the deletion was much larger than typically seen in SMS patients [Zori et al., 1993].

Our previous studies have shown the value of FISH in ruling out mosaicism for del(17)(p11.2p11.2) diagnosed during routine cytogenetic analysis [Juyal et al., 1995a,b]. This study illustrates the usefulness of FISH analysis in the diagnosis of mosaicism for del(17)(p11.2p11.2) overlooked by routine cytogenetic analysis. However, it should be noted that the frequency at which mosaicism for subtle chromosomal abnormalities goes undiagnosed is unknown. In our cohort of 62 SMS patients diagnosed cytogenetically as having an interstitial deletion in the short arm of chromosome 17, three were apparently mosaic for the deletion. Mosaicism was proven in one of these three patients by construction of somatic cell hybrids [Zori et al., 1993], and ruled out in the two others by FISH [Juyal et al., 1995a,b]. Forty-five of the sixty-two patients with a cytogenetic diagnosis of SMS were examined by FISH to determine the molecular extent of the deletion. This analysis demonstrated mosaisicism in one patient, HOU121-492, the subject of this report. Additional studies are required to determine if

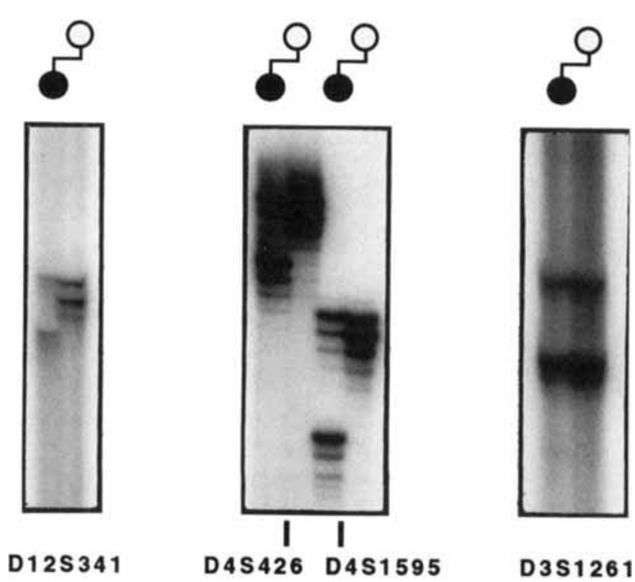

Fig. 2. Microsatellite genotypes at four different loci in SMS patient, HOU121-492, and her mother, HOU121-493. A maternal allele is present in the patient. The presence of only one or two alleles in HOU121-492 indicates that the cell line is not contaminated with a cell line with normal chromosome 17 homologues.

this frequency (1/45 or roughly 2%) of mosaic cases prevails in SMS and in other interstitial deletion syndromes.

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REFERENCES

Bunyan DJ, Crolla JA, Collins AL, Robinson DO (1995): Fluorescence in situ hybridisation studies provide evidence for somatic mosaicism in de novo dystrophin gene deletions. Hum Genet 95: 43–45.

Cassidy SB, Thuline HC, Holm VA (1984): Deletion of chromosome 15 (q11-13) in a Prader-Labhart-Willi syndrome clinic population. Am J Med Genet 17:485–495.

Chen K-S, Gunaratne PH, Hoheisel JD, Young IG, Miklos GLG, Greenberg F, Shaffer LG, Campbell HD, Lupski JR (1995): The human homologue of the Drosophila melanogaster flightless-I gene (fliI) maps within the Smith-Magenis microdeletion critical region in 17p11.2. Am J Hum Genet 56:175–182.

Chevillard C, Le Paslier D, Passage E, Ougen P, Billault A, Boyer S, Mazan S, Bachellerie JP, Vignal A, Cohen D, Fontes M (1993): Relationship between Charcot-Marie-Tooth 1A and Smith-Magenis regions. snU3 may be a candidate gene for the Smith-Magenis syndrome. Hum Mol Genet 2:1235–1243.

Dudin G, Nasr A, Traboulsi E, Khouri F, Der Kaloustian VM (1984): Hereditary retinoblastoma and 13-mosaicism. Cytogenet Cell Genet 38:235–237.

Elsea SH, Juyal RC, Jiralerspong S, Finucane BM, Pandolfo M, Greenberg F, Baldini A, Stover P, Patel PI (1995): Haploinsufficiency of cytosolic serine hydroxymethyltransferase in the Smith-Magenis syndrome. Am J Hum Genet 57:1342–1350.

Figuera LE, Pandolfo M, Dunne PW, Cantú JM, Patel PI (1995): Mapping of the congenital generalized hypertrichosis locus to chromosome Xq24-q27.1. Nature Genet 10:202–207.

Finucane BM, Jaeger ER, Kurtz MB, Weinstein M, Scott CI Jr. (1993a): Eye abnormalities in the Smith-Magenis contiguous gene deletion syndrome. Am J Med Genet 45:443–446.

Finucane BM, Kurtz MB, Babu VR, Scott CI Jr. (1993b): Mosaicism for deletion 17p11.2 in a boy with the Smith-Magenis syndrome. Am J Med Genet 45:447–449.

Finucane BM, Konar D, Haas-Givler B, Kurtz MB, Scott CI Jr. (1994): The spasmodic upper-body squeeze: a characteristic behavior in Smith-Magenis syndrome. Dev Med Child Neurol 36:78–83.

Gordon K, Siu VM, Sergovich F, Jung J (1993): 18q-mosaicism associated with Rett syndrome phenotype. Am J Med Gen 46:142–144.

Greenberg F, Guzzetta V, Montes de Oca-Luna R, Magenis RE, Smith ACM, Richter SF, Kondo I, Dobyns WB, Patel PI, Lupski JR (1991): Molecular analysis of the Smith-Magenis syndrome: A possible contiguous gene syndrome associated with del(17)(p11.2). Am J Hum Genet 49:1207–1218.

Juyal RC, Greenberg F, Mengden GA, Lupski JR, Trask BJ, van den Engh G, Lindsay E, Christy H, Chen K-S, Baldini A, Shaffer LG, Patel PI (1995a): The Smith-Magenis syndrome deletion: A case with equivocal cytogenetic findings resolved by fluorescence in situ hybridization. Am J Med Genet 58:286–291.

Juyal RC, Finucane B, Shaffer LG, Lupski JR, Greenberg F, Scott CI, Baldini A, Patel PI (1995b): Apparent mosiacism for del(17)(p11.2) ruled out by fluorescence *in situ* hybridization in a Smith-Magenis syndrome patient. Am J Med Genet 59:406–407.

Juyal RC, Figuera LE, Hauge X, Elsea SH, Lupski JR, Greenberg F, Baldini A, Patel PI (1996): Molecular analysis of 17p11.2 deletions in 62 Smith-Magenis syndrome patients. Am J Hum Genet 58: 998–1007.

Kondo I, Matsuura S, Kuwajima K, Tokashiki M, Izumikawa Y, Naritomi K, Niikawa N, Kajii T (1991): Diagnostic hand anomalies in Smith-Magenis syndrome: Four new patients with del(17) (p11.2p11.2). Am J Med Genet 41:225–229.

Michalová K, Kloucek F, Musilová J (1982): Deletion of 13q in two patients with retinoblastoma, one probably due to 13q-mosaicism in the mother. Hum Genet 61:264–266.

Romano C, Ragase RN, Scillato F, Greco D, Amato G, Barletta C (1991): Phenotypic and phoniatric findings in mosaic cri du chat syndrome. Am J Med Genet 39:391–395.

Smith ACM, McGavran L, Waldstein G, Robinson J (1982): Deletion of the 17 short arm in two patients with facial clefts and congenital heart disease. Am J Hum Genet 34:410A.

Smith ACM, McGavran L, Robinson J, Waldstein G, Macfarlane J, Zonona J, Reiss J, Lahr M, Allen L, Magenis E (1986): Interstitial deletion of (17)(p11.2p11.2) in nine patients. Am J Med Genet 24:393–414.

Stratton RF, Dobyns WB, Greenberg F, DeSana JB, Moore C, Fidone G, Runge GH, Feldman P, Sekhon GS, Pauli RM, Ledbetter DH (1986): Interstitial deletion of (17)(p11.2p11.2): Report of six additional patients with a new chromosome deletion syndrome. Am J Med Genet 24:421–432.

Voit T, Neuen-Jacob E, Mahler V, Jauch A, Cremer M (1992): Somatic mosaicism for a deletion of the dystrophin gene in a carrier of Becker muscular dystrophy. Eur J Ped 151:112–116.

Zhao Z, Lee C, Jiralerspong S, Juyal RC, Lu F, Baldini A, Greenberg F, Caskey CT, Patel PI (1995): The gene for a human microfibril-associated protein is commonly deleted in Smith-Magenis syndrome patients. Hum Mol Genet 4:589–597.

Zori RT, Lupski JR, Heju Z, Greenberg F, Killian JM, Gray BA, Driscoll DJ, Patel PI, Zackowski JL (1993): Clinical, cytogenetic, and molecular evidence for an infant with Smith-Magenis syndrome born from a mother having a mosaic 17p11.2p12 deletion. Am J Med Genet 47:504–511.